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# Thesis of Rachel Zimmerman

Submitted in Partial Fulfillment of the Requirements for the Degree of

## Master of Science M.S. Marine Biology

Nova Southeastern University  
Halmos College of Natural Sciences and Oceanography

August 2018

Approved:  
Thesis Committee

Major Professor: Bernhard Riegl

Committee Member: Dr. Robin Sherman

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

Genomic analysis of *Acropora cervicornis*  
mucus and sediments in the  
Florida Keys Tavernier Nursery

By

Rachel Zimmerman

Submitted to the Faculty of  
Halmos College of Natural Sciences and Oceanography  
in partial fulfillment of the requirements for  
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

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**Thesis of  
Rachel Zimmerman**

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**Marine Biology**

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**Committee Approval**

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Thank you to old friends and new for making me laugh and always lending an ear. The support you've given me has helped me finish and I look forward to future oh shit we're getting old shenanigans.

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## **Abstract**

White Band disease has devastated the staghorn coral *Acropora cervicornis* in recent decades, and it continues to impinge upon restoration efforts. The etiological agent(s) remain unknown as Koch's postulates have yet to be satisfied, but disease may originate when opportunistic pathogens in the surface mucus layer exploit a stressed host. Using 16s rRNA sequencing, differences in the taxonomic diversity and relative abundances of bacteria within the mucus of *A. cervicornis* were documented between colonies of the same genotype, genotypes (n=8) categorized as having either high or low WBD susceptibility, and during a transplantation event. *A. cervicornis* colonies suspended from midwater PVC trees via monofilament were sampled for mucus, after which half of the sampled colonies were relocated to the unconsolidated sediments below. Temporal changes in the microbiome of the pelagic and benthic corals were then monitored by sampling the same apical tip over time. Incidentally, all benthic colonies for this experiment became afflicted with WBD; thereby differences in healthy vs. diseased colonies and the effects of disease progression on the microbiome were documented. Water was sampled concurrently with all mucus experiments to resolve the degree of commonality in bacterial species between the two environments, and sediments were sampled in the transplant experiment to determine if sediments may act as a pathogen reservoir. In addition, sediment samples were collected to assess site and temporal differences in the benthic microbiome along a nearshore to offshore transect off Key Largo, Florida. Irrespective of the inclusion of water operational taxonomic units (OTUs), no differences between colonies of the same genotype were observed with regards to the bacterial communities sampled from mucus in either alpha diversity metrics [species richness, Shannon, Inverse Simpson] or phylogenetic relatedness as determined by weighted unique fraction (UniFrac) were detected between colonies. However, differences were observed in the Bray-Curtis dissimilarity matrices based on relative abundance and presence/absence of either [with and without water OTU] scenarios. Bacterial communities associated with different coral genotypes differed in species richness and Inverse Simpson in both water scenarios, as did weighted UniFrac and Bray-Curtis relative abundance and presence/absence transformed dissimilarity matrices. Alpha diversity of mucus bacteria was similar between corals of different disease-susceptibilities when water OTUs were either included or excluded, except for the Inverse Simpson index upon removal of water OTUs. Removal of aqueous bacteria also revealed significant differences between disease-susceptibility groups in Bray-Curtis relative abundance and presence/absence dissimilarity values that was not detected with the incorporation of water OTUs. Regardless of the presence of water OTUs, weighted UniFrac was similar between corals of different disease susceptibilities. Most notably, dispersion increased in the microbiome of coral genotypes with high disease susceptibility in all cases except for the relative abundance transformed Bray-Curtis dissimilarity matrix when water OTUs were incorporated. This finding is in accordance with the Anna Karenina Principle, which states that loss of microbial regulation leads to an unpredictable microbiome in diseased individuals. In the sediment experiment, location was the only factor influencing microbiome composition. These findings may be due to the short duration of the experiment and differences between the carbonate content of the sediments and hydrological regimes between sites.

**Keywords:** *Acropora cervicornis*, white band disease, coral restoration, microbiome, mucus, sediments, genotypes, disease susceptibility



## CHAPTER 1: INTRODUCTION

### 1.1 *Devastation of *Acropora cervicornis**

Average scleractinian cover in the Caribbean has plummeted by 80% (Gardner *et al.* 2003), with the once prominent reef-building species of this region - *Acropora cervicornis* - having suffered losses upwards of 98% in Florida alone (Miller, Bourque, and Bohnsack 2002). The extensive decline of *A. cervicornis* was caused predominately by White Band Disease (WBD; Aronson and Precht 2001; Aronson and Precht 2001a), with other contributing factors including hurricanes (Gardner *et al.* 2003), bleaching (Aronson *et al.* 2000), and the mass mortality of *Diadema antillarum* (Gardner *et al.* 2003; Lessios *et al.* 1984). As a result of herbivore loss and vast swaths of newly available skeletal substrate, reefs transitioned to coral recruitment-inhibiting algal-dominance (Aronson and Precht 2006). Local recovery of *A. cervicornis* may occur with WBD resistant genotypes, but regional recovery may hinge on successful sexual reproduction generating WBD-resistant gametes. However, the majority of Caribbean Reefs experience poor recruitment, and no studies have documented successful recovery via this method. Furthermore, larval dispersion is spatially constrained and thus repopulation is unlikely to occur on reefs beyond these larval limits (Vollmer and Kline 2008). *A. cervicornis* is now listed as threatened under the Endangered Species Act (National Marine Fisheries Service 2014) and as critically endangered under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Aronson *et al.* 2010). Restoration efforts have focused on restoring local populations (<http://www.coralrestoration.org/>) due not only to the precipitous decline in their numbers, but also due to the fact that this species grows relatively fast (Lirman *et al.* 2014), has a branching morphology that contributes to structural heterogeneity of the reef (Aronson and Precht 2001a), and asexually reproduces by means of fragmentation (Highsmith 1982). However, WBD remains a sustained threat to the persistence of outplanted colonies (Miller *et al.* 2014), and successful restoration hinges upon outplanting a wide variety of WBD-resistant genotypes (Vollmer and Kline 2008).

### 1.2 *White band disease*

First recognized in 1979, WBD only infects the *Acroporid* corals *A. cervicornis*, *A. palmata* (Gignoux-Wolfsohn, Marks and Vollmer 2012; Gignoux-Wolfsohn and

Vollmer 2015), and their F1 hybrid *A. prolifera* (Polson 2007). It is characterized by a progressing band of denuded calcium carbonate skeleton that is further characterized depending on if bleaching precedes tissue loss (type II) or not (type I). While type II can resemble type I by originating at the base of the colony and proceeding towards the apical branch tip, type II can also originate at the apical tip and proceed towards the base. Moreover, the band of bleached tissue in type II can disappear if bleaching ceases and the sloughing band converges on the zooxanthellate tissue, thereby mimicking type I disease signs. A *Vibrio charchariae*-like bacterium is the suspected agent of WBD II (Ritchie and Smith 1998), but the etiological agent(s) of WBD I remain(s) unknown (Kline and Vollmer 2011; Sweet *et al.* 2014; Gignoux-Wolfsohn, Marks and Vollmer 2012; Gignoux-Wolfsohn and Vollmer 2015). Antibiotic experiments suggest a bacterial origin (Kline and Vollmer 2011; Sweet *et al.* 2014) with *Vibrio charchariae*, *Bacillus* sp. and *Lactobacillus suebicus* as suspected agents (Sweet *et al.* 2014; Gignoux-Wolfsohn and Vollmer 2015). The 16S rRNA sequences of the two former species is 100% homologous to the WBD type II pathogen detected in Puerto Rico and the potential causative agent of Acroporid WBD in Indonesia, respectively (Sweet *et al.* 2014). Field-sampled and disease inoculated nubbins suggest *Flavobacteriales* species may be a potential culprit (Gignoux-Wolfsohn and Vollmer 2015), while histological analysis suggests a *Rickettsiales* species (Peters 2014). However, Casas *et al.* (2004) discovered a coral-associated *Rickettsiales* 1 (CAR1) bacterium in both healthy and diseased samples, and laser capture microdissection of suspected *Rickettsiales* aggregates in the tissues of diseased *A. prolifera* resembled *Pseudomonas mendocina* (Polson 2007).

WBD is transmissible via the water column if the coral has sustained previous damage (Gignoux-Wolfsohn and Vollmer 2015) and by the predatory corallivorous snail - *Coralliophila abbreviata* (Gignoux-Wolfsohn, Marks and Vollmer 2012; Gignoux-Wolfsohn and Vollmer 2015). In addition to likely weakening coral immunity while simultaneously increasing pathogen virulence (Pollock *et al.* 2014; Sheridan *et al.* 2014), sediments may also be a vector by which bacterial pathogens are transferred from the benthos to corals (Pollock *et al.* 2014; Hodgson 1990). Tetracycline-treated water diminished tissue necrosis of corals exposed to sedimentation (Hodgson 1990), and Sheridan *et al.* (2014) were able to induce characteristic signs of white pox disease by exposing *Acropora palmata* to *Serratia*

*marcescens*-adsorbed sediment particles. Of the several potentially pathogenic bacterial strains these authors isolated from the sediments, one included the suspected etiological agent of white syndrome - *Vibrio harveyi* (Sheridan *et al.* 2014). Furthermore, sediment originating from dredging and terrestrial outflow increased the prevalence of coral disease in Australian and Madagascan reefs respectively (Pollock *et al.* 2014; Sheridan *et al.* 2014), and - in Australia - was also correlated with necrosis, bleaching, and anomalous pigmentation of corals (Sheridan *et al.* 2014). Likewise, corals at heavily frequented dives sites had a greater incidence of tissue necrosis stemming from sedimentation than did less popular dive sites, and the percent occurrence of sediment-linked tissue necrosis was significantly associated with white syndromes (Lamb *et al.* 2014).

### 1.3 Surface mucus layer

WBD may arise in stressed corals if the altered surface mucus layer (SML) becomes vulnerable to invasion by opportunistic pathogens, initiating a community shift to a less desirable *Vibrio*-dominated state (Ritchie 2006). Stress usually refers to bleaching, which has been linked to WBD outbreaks (Randall and van Woesik 2015) and changes in the sugar content of the SML that make it more conducive to pathogen growth (Randall and van Woesik 2015, Lee *et al.* 2016). The SML is the first defense against pathogens (Brown and Bythell 2005, Krediet *et al.* 2009), and functions in nutrient procurement, prevention of sediment accumulation and desiccation, UV protection (Toledo-Hernández 2014; Ritchie 2006; Brown and Bythell 2005; Kellogg 2004), and molecular exchange between the surrounding aqueous environment and underlying tissue (Brown and Bythell 2011). Commensal bacteria within this layer confer adaptive immunity to their host (Ritchie 2006, Polson 2007, Koenig *et al.* 2011) by synthesizing numerous compounds detrimental to harmful bacteria such as antibiotics, antibiotic secretion inhibitors, (Ritchie 2006), molecules that preclude biofilm production and swarming behavior, and compounds that interfere with cell-to-cell communication (Krediet *et al.* 2013). Bacteria can also inhibit the spread of disease (Alagely *et al.* 2011). However, some bacteria may become opportunistically pathogenic (Certner and Vollmer 2015) during periods of thermal stress and may suppress a coral's defensive capabilities while simultaneously heightening virulence of opportunistic pathogens (Harvell *et al.* 2007). Furthermore, interactions between the host, pathogens, and symbiotic counterparts may additionally be modulated by

thermal stress (Harvell *et al.* 2007). Alternatively, mucus pathogens may proliferate by thwarting commensal antibiotics with mobile genetic elements ('cassettes') encoding acetyltransferases (Koenig *et al.* 2011), and/or by preferentially hydrolyzing different mucus constituents which may then mediate future mucus metabolism by differently affecting enzyme gene regulation in each microbe. Pathogens may employ different enzymes, differential enzyme regulation and activity (Krediet *et al.* 2009 and 2009b), and/or may encourage colonization by other harmful species via kin selection (Ritchie 2006).

#### 1.4 Microbiome dynamics

As WBD continues to afflict transplanted colonies (Miller *et al.* 2014) and microbes linked to coral disease increase when colonies are transplanted (Pratt, Richardson, and DeEtta 2015, Casey *et al.* 2015), microbiome research in this area may advance restoration efforts by assisting in identifying new areas of investigation related to WBD. Given that only 0.01-1% of marine bacteria are cultivatable by traditional methods (Polson 2007; Amann *et al.* 1995), culture-independent methods such as 16S rRNA sequencing have become increasingly popular for documenting marine microbiomes. 16S rRNA is an approximately 1,550 bp sequence that is a constituent of the small subunit of the protein-synthesizing ribosome (Woese *et al.* 1983; Clarridge III 2004), and is frequently used as a taxonomic determinant because of its ubiquity and hypervariable regions (V1-V9) unique to each species (Polson 2007; Clarridge III 2004, Wahl *et. al.*). These variable regions are distinctive to each species since they are prone to random mutations resulting from minimal evolutionary pressure, and the conservative regions with which they are interspersed permit the use of general primers for DNA amplification (Polson 2007; Clarridge III 2004, Wahl *et. al.*).

This study investigated differences in the SML microbiome of *A. cervicornis* by sequencing the V4 region of the 16s rRNA gene. By collaborating with the Coral Restoration Foundation (CRF) in Key Largo, Florida, the available materials allowed comparisons to be made between individual coral colonies, between genotypes of either high or low WBD susceptibilities, and between colonies relocated from mid-water suspended PVC trees to placement on the unconsolidated sediments directly below their tree of origin. Although colonies are usually secured to the benthos by epoxy, in this

experiment corals were left unaltered. Additionally, water was sampled once during the course of the experiment to assess the extent of bacterial commonality between the microbiomes of mucus and seawater. Also, the microbiome of sediments was sampled adjacent to the transplanted corals to detect what, if any, role sediments play in WBD. Lastly, sediments were sampled at four different sites and at two different dates to uncover site- and time-specific differences within the Florida Keys.

## **CHAPTER 2: METHODS AND MATERIALS**

### *2.1 General overview of coral nursery*

All coral mucus sampling was conducted at the Coral Restoration Foundation's Tavernier Nursery located off Key Largo, Florida (24° 58.940'N, 80° 26.187'W). In the nursery, corals are suspended via monofilament to the branches of PVC trees that are buoyed to float off the seafloor to midwater depths of approximately six meters; each tree contains ramets which were identified with regards to their genotypic classification by a tag secured to the tree. Colonies utilized in this experiment originated as small fragments that were reared to a maximum diameter greater than ten cm over approximately twelve months. Genotype designations and phenotypic characterizations relevant to disease susceptibility were provided by CRF staff, whom determine disease ranking through routine monitoring of disease at the nursery and at outplant sites (Amelia Moura, pers.comm.).

### *2.2 Intercolony patterns in bacterial SML communities*

Mucus from four apical tips of *A. cervicornis* was sampled on August 6, 2016 from five different apparently healthy [devoid of outward signs of disease] colonies of the M5 genotype. Mucus sloughing was induced by gently irritating the epidermis of each apical tip using the sterile end of a 10 mL syringe (Global Medical Supply). Colonies were arbitrarily designated as A, B, C, D, and E.

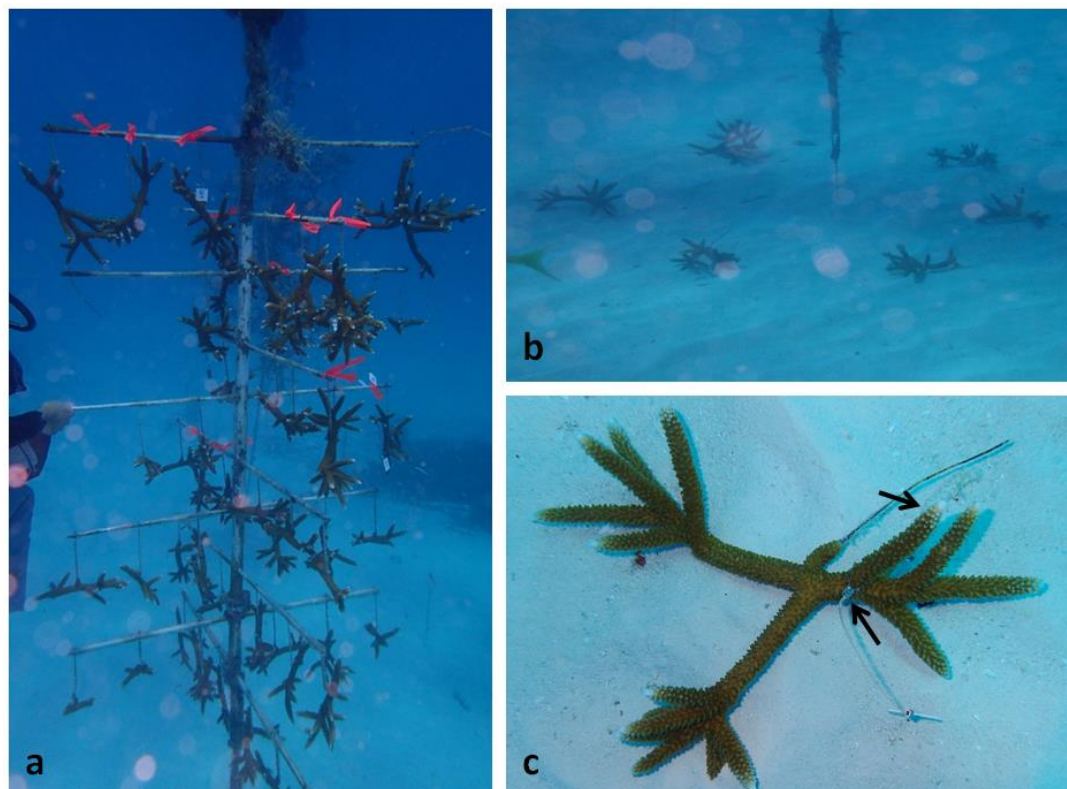
### *2.3 Sampling dependent on genotype and known disease-susceptibility*

Mucus from nine genotypes with either high (U7, U8, U29, U44, U46) or low (M3, U47, U54) disease susceptibility were aspirated into 10 mL syringes (Global Medical Supply) on August 7, 2016 by gently agitating one apical tip per colony of each genotype; four colonies were sampled per genotype. In an attempt to reduce the

inadvertent aspiration of tissue and/or skeleton when using a syringe tip, tips in this and subsequent experiments were instead irritated with sterile latex examination gloves (Dynarex).

#### 2.4 Temporal differences in colonies relocated from midwater trees to the sandy benthos

One coral tree each with high (K2) and low (M5) disease susceptibility (as predetermined by CRF staff) were selected based on the absence of diseased colonies. For each genotype, twelve corals of approximately the same size were selected for sampling by measuring their maximum primary and secondary branch diameters and maximum branch height as described in Miller *et. al.*(2013). After initial mucus sampling utilizing the protocol outlined above, each colony of which the tip was sampled was tagged at the base of the branch. Six colonies were left on the tree, and the other six were clipped and placed on the sediments below in a circle surrounding the tree with the tip sampled for mucus facing skyward ('Day 1' - 8-4-16)(Fig. 1).



**Figure 1.** Experimental transplant setup a. Twelve tree corals of approximately equivalent sizes were selected for each of the two genotypes (M5 and K2). After mucus sampling, six of the corals were transferred to the benthos and arranged in a circular pattern with the mucus tip most distal to the sediments b. base of labeled branch (left arrow) whose apical tip (right arrow) was repeatedly sampled for mucus

Over the course of the experiment, disease presence, disease type, location of disease origin (sediment/non-sediment), and percent coral mortality (living versus dead tissue) were documented. The same tip previously sampled for mucus was subsequently sampled for mucus on days 2, 5, 9, 14, and 21, with healthy colonies sampled prior to diseased individuals in an effort to minimize the chance of disease transmission.

To determine the extent of microbial contributions made to the mucus microbiome of the corals in this experiment, sediments located between the two trees were collected into 15-mL Falcon tubes (Corning, Inc.) on days 9 (August 12, 2016) and 21 (August 24, 2016). Additionally, a midwater and benthic water sample was collected with HCl-sterilized 1 L Nalgene bottles on day 9 (August 12, 2016).

## *2.5 Sediment transect*

On August 12, 2016 and September 29, 2016, sediments along a nearshore to offshore transect were sampled in Key Largo, FL using 15-mL Falcon Tubes (Corning, Inc.) at four different locations: Tavernier Creek (24°59.441 -80°31.415), Hawks Channel (24°59.114 -80°28.441), Tavernier Nursery (24° 58.940'N, 80° 26.187'W), and the rubble patch adjacent to Pickles Reef (24°58.970 -80°25.168).

## *2.6 Sample processing and sequencing*

Mucus contained within the syringes was dispensed into 15-mL Falcon tubes (Corning, Inc.) and along with sediment samples, submerged in a -80°C dry ice/ethanol slurry. Water samples were kept on ice until the contents were 0.45 µm filtered, with the filters subsequently stored in 2 mL Eppendorf tubes in a dry ice/ethanol slurry. All samples remained in slurry until stored at -80°C.

Samples collected for the colony and genotype experiments were centrifuged at 2370 RCF for 10 minutes to pellet the mucus, with the resulting supernatant discarded. Since a standard centrifuge speed of  $15000 \times g$  for 10 min was required for mucus samples and a competent centrifuge was not available to accommodate the 15 mL tube and speed, transplant mucus samples were portioned out into 1.5 mL Eppendorf tubes to centrifuge at the recommended speed. DNA for sediment, colony, and genotype mucus samples (M3, U7, U8) were extracted with the DNeasy PowerLyzer PowerSoil kit (Cat# 12855-100). DNA of the remaining genotype (U29,

U44, U46, U47, U54) and transplant mucus samples were extracted with the QIAamp BiOstic Bacteremia DNA kit (Cat# 12240-50) (MoBio Laboratories Inc.) once it was discovered this kit achieved higher DNA yields.

Barcoded universal primers 806R (5'- GGACTACHVGGGTW TCTAAT- 3') and 515F (5'- GTGCCAGCMGCCGCGGTAA- 3') specifically fabricated to hybridize to the conservative region adjoining the V4 hypervariable region of the 16S rRNA subunit were used to amplify this region via PCR (Cuvelier *et. al.* 2014, Caporaso *et. al.* 2011). PCR solutions were comprised of the following reagents: 1.0 µL sample DNA, 13 µL PCR-grade water, 10 µL 5Prime HotMaster Mix, and 0.5 µL of forward and 0.5 µL of reverse primers. DNA was denatured by maintaining the solution at 94°C for 3 mins, followed by 35 amplification cycles of 94°C for 45s, 50°C for 60s, and 72°C for 90s, and concluded with a quality assurance step at 72°C for 10 min (Caporaso *et. al.* 2011). Amplicons were then intercalated with GelRed™ prior to visualization on a 1.5% agarose gel to assure that the PCR products were approximately 254 bp in length. The amplicons were purified with an Agencourt AMPure XP kit prior to quantification with a Qubit Fluorometer and dilution to 4 nM. Samples were then pooled, denatured with sodium hydroxide, diluted, and loaded into a 500-cycle V2 cartridge for sequencing with the Illumina MiSeq (Illumina Corp). Barcodes and primers were cleaved subsequent to sequence output.

## 2.7 Post sequence processing

Using the barcode information within the mapping file, demultiplexing was performed as described in Quantitative Insights into Microbial Ecology (QIIME) version 1.9.1. by linking sequences to the samples from which they originated. Demultiplexing means that every bacteria within a sample was labeled with the same unique nucleotide sequence ('barcode') to allow identification from which sample the bacteria originated from. After subtracting the barcode and V4 primer, the remaining sequence was the V4 region of the ribosome. Due to a decline in the quality at the terminal end of the reverse read (R2), only forward reads with a Phred score greater than 30 were included in the analysis. Operational taxonomic units (OTUs) were determined by open-reference clustering the quality-filtered sequences, and the average ('centroid') sequence representing each cluster was designated by the USEARCH algorithm. Taxonomy was assigned by aligning sequences against those



contained within the SILVA database with a 97% homology threshold using the RDP classifier approach with a 0.8 confidence level; a phylogenetic tree was generated using the FastTree method. Upstream analysis culminated in an OTU table constructed by the Genomics Standards Consortium's Biological Observation Matrix (BIOM).

## 2.8 Statistical analysis

Prior to statistical analysis in R (<https://www.r-project.org/>) using the 'vegan' (Oksanen *et al.* 2013) and 'picante' (Kembel *et al.* 2010) packages, sequences identified as chloroplasts (Zaneveld *et al.* 2016) and singletons (Glasl *et al.* 2016) by the converted BIOM file were excluded from analysis. Singletons are sequences observed only once in a data set, and are attributable to sequencing artifacts (Achaz 2008). Rarefaction curves were generated to assess whether sampling depth was sufficient to accurately characterize the community. If species numbers displayed logarithmic growth, more intensive sampling was necessary and samples were therefore discarded; curves that plateaued indicated sampling effort was sufficient to accurately represent the totality of species within the sample (Navas-Molina *et al.* 2013). Datasets excepting sediments were evaluated with and without the combined water OTUs, as water was not sampled at all sediment collection sites.

Alpha diversity details species diversity within a sample, and is predicated on the number (species richness) and/or comparative frequency (species evenness) of different species contained within each sample. For this experiment, richness was ascertained with an OTU count, and both richness and evenness were determined by the Shannon diversity and Inverse Simpson Indices. To determine if each of the three diversity measures were different among sample groups, group means were compared with one another using an Analysis of Variance (ANOVA) test. If ANOVA was significant, a post hoc Tukey's Honest Significant Difference Test was used to compare samples pairwise to determine which samples differed from one another (Keselman *et al.* 1998).

Beta diversity describes species diversity between samples (Lozupone and Knight 2008), and was characterized in this experiment using Bray-Curtis dissimilarity matrices and weighted UniFrac distances. Bray-Curtis detects compositional dissimilarity between samples by calculating shared species (Urban *et al.* 2002), and

data for this statistic was transformed into presence/absence (PA) and relative abundance (RA) to uncover differences that may not be discernible if only using one transformation (Lozupone and Knight 2008). Weighted UniFrac is likewise a pairwise comparison, but differs in that it assimilates a phylogenetic tree to determine branch lengths exclusive to each sample. Furthermore, each fraction of length is weighted according to relative abundance (Navas-Molina et al 2013; Zaneveld *et al.* 2016). To ascertain if differences existed, an Adonis test was administered. Adonis determines if the distance to a centroid is greater between than within groups by calculating an F-statistic, and significance is then enumerated by comparing the F-statistic to a distribution generated from random permutations (Daniels *et al.* 2011). If significant, a pairwise PERMANOVA was applied to confirm which samples differed from one another (Wilson et al. 2014). As equal variances are a prerequisite of PERMANOVA (Daniels *et al.* 2011), variance to a sample's centroid was calculated with the R function "betadisper" (Oksanen *et al.* 2013). Differences in variance between samples were tested with an ANOVA, followed by a Tukey Honest Significant Difference Test upon discovery of significance.

Pairwise comparison of matrix values can be translated into a distance matrix that can be visualized using ordination metrics. Samples clustering tightly together indicate high similarity with one another, while samples further apart indicate differences between communities (Zaneveld, McMinds, and Vega Thurber 2017). Bray-Curtis dissimilarities and weighted UniFrac were visualized with non-metric multidimensional scaling, with stress values providing goodness of fit.

OTUs from the mid- and benthic water samples were coalesced, and OTUs with relative abundances in excess of 1% were screened against samples from each of the three mucus experiments to determine which OTUs were common to each environment. The relative abundances of the shared OTUs in mucus were summed to establish what percentage of total mucus OTUs were comprised of those also contained within the overlying water. Additionally, these shared OTUs were subjected to a PERMANOVA in order to elucidate if they grouped according to the variable in question.

## CHAPTER 3: RESULTS

### 3.1 Intercolony patterns in bacterial SML communities

With the inclusion of sequences also detected in water, a total of 4220 OTUs were discovered in colony mucus samples. For alpha diversity, species richness was indistinguishable between colonies (ANOVA  $p=0.963$ ), while metrics incorporating both richness and evenness were borderline significant (ANOVA  $p=0.0503$  performed on Shannon index) or nearly so (ANOVA  $p=0.0664$  performed on Inverse Simpson). Although ANOVA results for both statistics indicated differences, a Tukey Test of Shannon and Inverse Simpson indices suggested no significant differences when colonies were compared pairwise (Table 1). For beta diversity as determined from Bray-Curtis matrices, colonies had dissimilar relative abundances in OTUs (Adonis test  $p=0.002$ ) and which species were present (Adonis  $p=0.002$ ); a test for multivariate homogeneity of group dispersions (R function ‘betadisper’) confirmed that the prerequisite of equal dispersions was satisfied for Adonis (RA ANOVA  $p=0.4136$ , PA ANOVA  $p=0.4829$ ). When a false discovery rate (FRD)-adjusted Pairwise Permanova was administered to discern differences responsible for Adonis significance, no between colony differences were identified. For weighted UniFrac, all colonies hosted microbial communities with similar kinship (Adonis  $p=0.935$ ), and their phylogenetic dispersions calculated from betadisper did not significantly differ (ANOVA  $p=0.1162$ ).

**Table 1.** Tukey’s Honest Significant Difference Test of Shannon and Inverse Simpson indices for colony mucus samples with all (water included) OTUs \* denotes nearly significant values

Diversity Metric	OTUs	> Colony	< Colony	p-value
Shannon	All	B	A	0.0601781*
			E	0.0718595*
Inverse Simpson	All	B	A	0.0866563*
			E	0.0720398*

When water OTUs with a relative abundance in excess of 1% were juxtaposed with colony mucus OTUs, 1296 OTUs coincided; the relative abundances of these OTUs averaged across colony samples was 89.36%. The relative abundance of the shared OTUs was influenced by colony (Adonis  $p=0.004$ ), as was species presence/absence (Adonis  $p=0.007$ ).

Upon excision of the OTUs in common with water, the number of colony-specific sequences was 2924 OTUs. Species richness was similar between colonies (ANOVA  $p=0.947$ ), as was richness and evenness as determined by Shannon (ANOVA  $p=0.516$ ) and Inverse Simpson (ANOVA  $p=0.65$ ). Bray Curtis relative abundance and presence/absence were significant (both Adonis  $p=0.001$ ), but an FDR-adjusted Pairwise Permanova was unable to expound which colonies contributed to observed differences. For both Bray-Curtis matrices, centroidal variance between colonies as ascertained by betadisper were similar (RA ANOVA  $p=0.7681$  and PA ANOVA  $p=0.4376$ ). Weighted branch lengths unique to each colony weren't significantly different from one another (weighted UniFrac Adonis  $p=0.667$ ), nor was phylogenetic variance around each colony's centroid (Betadisper ANOVA  $p=0.8467$ ).

### 3.2 *Genotype and susceptibility*

The number of comprehensive genotype OTUs in mucus samples was  $n=5807$ . Significant differences in alpha diversity between genotypes was established with species richness (ANOVA  $p=0.00523$ ) and Inverse Simpson (ANOVA  $p=0.0164$ ), but not for Shannon (ANOVA  $p=0.323$ )(Table 2). According to values obtained from the Bray-Curtis dissimilarity matrices, genotypes hosted bacteria in not only different relative abundances (Adonis  $p=0.001$ ), but different species were present as well (Adonis  $p=0.001$ ). Although genotypic differences with both Bray-Curtis dissimilarities were not mirrored with an FDR-adjusted Pairwise Permanova, the assumption of equal variances was verified with betadisper (RA ANOVA  $p=0.9101$  and PA ANOVA  $p=0.1322$ ). Values for weighted UniFrac differed by genotype (Adonis  $p=0.001$ ), but differences were not substantiated with a Pairwise Permanova. Moreover, mean centroidal distances were similar between genotypes (Betadisper ANOVA  $p=0.1154$ ). When genotypes were dichotomized according to WBD susceptibility, no alpha diversity discrepancies were present between high and low (ANOVA species richness  $p=0.307$ , ANOVA Shannon  $p=0.153$ , ANOVA Inverse Simpson  $p=0.149$ ). Likewise, categorization by susceptibility did not affect bacterial relative abundances (Bray-Curtis Adonis  $p=0.245$ ), which species were present (Bray-Curtis Adonis  $p=0.148$ ), or unique lineages weighted by relative abundance (weighted UniFrac Adonis  $p=0.648$ ). Susceptibility did not influence variance around the Bray-Curtis relative abundance centroid (betadisper ANOVA  $p=0.4372$ ), but the

assumption of equal dispersions was violated for presence/absence (betadisper ANOVA  $p=0.02619$ ) and weighted UniFrac (betadisper ANOVA  $p=0.001462$ ). In both significant instances, high susceptibility had more dispersion than low (Tukey's Test PA  $p=0.0261854$ , Tukey's Test weighted UniFrac  $p=0.0014621$ ).

**Table 2.** Alpha diversity metrics (Richness, Shannon, Inverse Simpson) for mucus samples obtained from eight different genotypes with all (water included) and genotype-specific (water excluded) OTUs \*denotes nearly significant values

Diversity Metric	OTUs	> Genotype	< Genotype	p-value
Richness	All	U44	U7	0.0161839
			U8	0.0047501
		U54	U8	0.0658035*
	Specific	U44	U7	0.0301853
			U8	0.0382552
Shannon	Specific	U54	U7	0.0862509*
		M3	U47	0.0730546*
		U8	U44	0.0611373*
			U47	0.0199423
		U29	U47	0.0517642
		U46	U7	0.0052368
			U44	0.0002493
Inverse Simpson	All		U47	0.0000730
			U54	0.0186604
	Specific	U47	U8	0.0373397
			U46	0.0748036*
		U46	M3	0.0171365
			U7	0.0013781
			U8	0.0270636
			U29	0.0212687
			U44	0.0002248
			U47	0.0000642
			U54	0.0003505

With  $n=1760$  water OTUs coinciding with those found in mucus samples, the relative abundances of these shared OTUs averaged across all coral genotype samples was 92.623%. The relative abundance and presence/absence of shared OTUs significantly depended on coral genotype (Bray-Curtis RA Adonis  $p=0.001$  and Bray-Curtis PA  $p=0.001$ ) but not WBD susceptibility classification (Adonis  $p=0.423$  and Adonis  $p=0.312$ , respectively).

The number of coral genotype-specific OTUs was  $n=4137$ , with coral genotype significantly affecting species richness (ANOVA  $p=0.0164$ ), Shannon (ANOVA  $p=9.13e-05$ ), and Inverse Simpson (ANOVA  $p=9.41e-05$ ). All matrices designated by genotype were significant (Bray-Curtis RA Adonis  $p=0.001$ , Bray-Curtis PA Adonis  $p=0.001$ , weighted UniFrac Adonis  $p=0.001$ ); Pairwise Permanova was significant for Bray-Curtis RA only (Table 3). Although dispersions were similar between coral

**Table 3.** Pairwise Permanova results of relative abundance transformed Bray-Curtis dissimilarity values for eight different *A. cervicornis* genotypes; water OTUs were excluded in the analysis

	M3	U29	U44	U46	U47	U54	U7
U29	0.381	-	-	-	-	-	-
U44	0.050	0.050	-	-	-	-	-
U46	0.050	0.159	0.050	-	-	-	-
U47	0.053	0.050	0.050	0.050	-	-	-
U54	0.073	0.083	0.050	0.050	0.102	-	-
U7	0.050	0.050	0.050	0.050	0.050	0.050	-
U8	1.000	0.159	0.050	0.159	0.050	0.050	0.050

genotypes for Bray-Curtis PA (Adonis  $p=0.2407$ ), dispersions were significantly different between genotypes for Bray-Curtis RA (Adonis  $p=9.108e-06$ ) and weighted UniFrac (Adonis  $p=0.01588$ ; Fig. 2), thus violating the assumption of equal variances; genotypes differing in their dispersions for Bray-Curtis RA and weighted UniFrac were discovered with a Tukey HSD (Table 4). Neither species richness (ANOVA  $p=0.46$ ) nor Shannon diversity (ANOVA  $p=0.121$ ) differed between susceptibility groups. Susceptibility did however influence Inverse Simpson diversity (ANOVA  $p=0.0521$ ), and a Tukey test indicated Inverse Simpson diversity was greater for the high susceptibility group than for the low susceptibility group ( $p=0.0520925$ ). Both Bray-Curtis dissimilarity matrices were significant for susceptibility (RA Adonis  $p=0.006718$ , PA Adonis  $p=8.253e-05$ ), although weighted UniFrac was not (Adonis  $p=0.005726$ ). However, the dispersion for genotypes with high disease susceptibility was greater than the dispersion seen in genotypes of low susceptibility for all three Adonis statistics (Bray-Curtis RA betadisper  $p=0.01$ , Bray-Curtis PA betadisper  $p=0.001$ , weighted UniFrac betadisper  $p=0.006$ ).

**Table 4.** Results of Tukey's HSD for betadisper of Bray-Curtis relative abundance and weighted UniFrac for coral genotype-specific OTUs \* denotes nearly significant values

Tukey HSD	> genotype	< genotype	p-value
Betadisper RA	M3	U44	0.0033308
		U47	0.0103050
	U8	U44	0.0003305
		U47	0.0010482
	U29	U44	0.0102523
		U47	0.0303939
	U46	U7	0.0269200
		U44	0.0000525
		U47	0.0001640
		U54	0.0253090
Weighted UniFrac	U46	U44	0.0178104
		U47	0.0551863*

### 3.3 Sediment transect

Due to integer constraints imposed by R-Studio,  $n=78,250$  sediment OTUs were pared down to  $n=23,169$  (55,081 OTUs excluded) by only including OTUs whose relative abundances were in excess of 0.01%.

**Table 5.** Alpha diversity metrics (Richness, Shannon, Inverse Simpson) for sediment samples obtained from four different sites along a nearshore to offshore transect off of Key Largo, FL \* denotes nearly significant values

Diversity metric	> Site	< Site	p-value
Richness	Hawks Channel	Tavernier Nursery	0.0365476
Shannon	Hawks Channel	Tavernier Creek	0.0065745
	Pickles Rubble Patch	Tavernier Creek	0.0116217
Inverse Simpson	Hawks Channel	Tavernier Creek	0.0031784
	Pickles Rubble Patch	Tavernier Creek	0.0089958
	Tavernier Nursery	Tavernier Creek	0.0659238*

Location affected all alpha diversity parameters: species richness (ANOVA  $p=0.0514$ ), Shannon diversity (ANOVA  $p=0.0522$ ), and Inverse Simpson diversity (ANOVA  $p=0.00296$ )(Table 5), whereas time (and its interaction with location) did not. Bray-Curtis dissimilarity calculated from RA transformed data was affected only by location (ADONIS  $p=0.001$ ), as was presence/absence transformed Bray-Curtis (ADONIS  $p=0.001$ ) and weighted UniFrac (ADONIS  $p=0.001$ ); a Pairwise Permanova was applied to determine which locations differed from one another (Table 6). Between sediment samples, Bray-Curtis relative abundance dispersions were indistinguishable from one another when analyzed by location (betadisper

**Table 6.** Pairwise Permanova for Bray-Curtis RA, PA and weighted UniFrac for four different sites along a nearshore to offshore transect off of Key Largo, FL

<u>Bray-Curtis RA</u>			
	Hawks Channel	Pickles Reef	Tavernier Creek
Pickles Reef	0.0045	-	-
Tavernier Creek	0.0050	0.0045	-
Tavernier Nursery	0.0045	0.0050	0.0045
<u>Bray-Curtis PA</u>			
	Hawks Channel	Pickles Reef	Tavernier Creek
Pickles Reef	0.0045	-	-
Tavernier Creek	0.0050	0.0045	-
Tavernier Nursery	0.0045	0.0050	0.0045
<u>Weighted UniFrac</u>			
	Hawks Channel	Pickles Reef	Tavernier Creek
Pickles Reef	0.0045	-	-
Tavernier Creek	0.0050	0.0045	-
Tavernier Nursery	0.0045	0.0050	0.0045

ANOVA  $p=0.1119$ ), day (betadisper ANOVA  $p=0.7309$ ), and their interaction (betadisper ANOVA  $p=0.3552$ ). While variance was equal for Bray-Curtis PA by site (betadisper ANOVA  $p=0.885$ ) and the interaction of site and date sampled (betadisper ANOVA  $p=0.2464$ ), variance was unequal when location was taken into account (betadisper ANOVA  $p=0.01465$ ). When a Tukey HSD was applied for the latter, Pickles Reef had greater dispersion than Tavernier Nursery ( $p=0.0376$ ). Like Bray-Curtis PA, weighted UniFrac was affected only by location (betadisper ANOVA  $p=0.03034$ ) and not day sampled (betadisper ANOVA  $p=0.7429$ ), or their interaction (betadisper ANOVA  $p=0.3803$ ). According to a Tukey HSD test, Pickles Rubble Patch had more variation around the weighted UniFrac centroid than did Tavernier Creek ( $p=0.0346$ ).



## CHAPTER 4: DISCUSSION

### 4.1 *Mucus regulatory mechanisms*

Colonies had essentially no significant differences in bacterial alpha diversity metrics, but they did for beta diversity. Bacteria in the mucus from different M5 colonies had unequal relative abundances and species present according to Bray-Curtis when water OTUs were both included and excluded. These findings suggest colonies within the same genotype differed in their microbial regulatory ability and/or colonies were exposed to different environmental conditions. These results align with the findings of Hernandez-Agrede *et al.* (2016), who catalogued three associations within the coral microbiome of a generalist species: a restricted universal core encountered irrespective of other influencing factors, a consortium routinely found in corals occupying a distinct habitat, and an ephemeral assemblage sensitive to local and regional environmental fluctuations. The authors argue that the preponderance of the most variable fraction is retained within the mucus due to constant fluctuations at a micro- and macroscale (Hernandez-Agrede *et al.* 2016). However, colonies may have differing regulatory mechanisms, as all of the corals in this experiment likely experienced somewhat homogenous conditions due to residing on the same tree and colonies having a maximum depth difference of only 0.9 m. While pelagic microbiomes can vertically stratify (Ghiglione *et al.* 2008), the depths at which they did so far exceeded the nominal depth difference in this study. Moreover, while *Orbicella annularis* microbiomes varied at depths of 5, 10, and 20 m, the monosaccharide and amino acid constitution of mucus did not (Klaus *et al.* 2007). Since the mucus microbiomes of corals change upon alteration of its sugar constituents (Lee *et al.* 2016), compositional consistency presumably lends to a stable mucus microbiome. Proximity to turf algae may also have influenced observed differences, as the microbiome of *Orbicella annularis* tissue abutting turf algae drastically changed to include more pathogenic bacteria (Barott *et al.* 2012).

Differences were also documented in other studies, both intra- and intercolony. A ribotype present in tip samples of *Porites furcata* fragments was absent in slurry obtained from the middle branch portion (Rohwer *et al.* 2002). Moreover, the mucosal microbiome of three *Montastraea annularis* colonies differed by a mean of 31% and the variability of within colony dissimilarities ranged from 9-61%. Up to 25% of this

variability was due to the different relative abundances of three OTUs common to all mucus samples (Daniels *et al.* 2011). Mucus from *A. palmata* collected from three spatially distinctive patches (base, skyward facing and underside) was homogenous, and no significant variance was detected in the microbiomes of different colonies (Kemp *et al.* 2015). The differences between the mucus of these coral species may be due to the assortment of gastrodermal zooxanthellae, since photosynthetically-derived products may influence its microbial community when transferred to the SML. Indeed, *M. annularis* hosts a diverse community of zooxanthellae whereas *A. palmata* usually hosts one strain (Kemp *et al.* 2015). While *A. cervicornis* can simultaneously harbor different zooxanthellae clades, one clade routinely dominates (Lirman *et al.* 2014). As such, the mucus microbiome of *A. cervicornis* would presumably be homogenous and in contradiction with the results of this experiment.

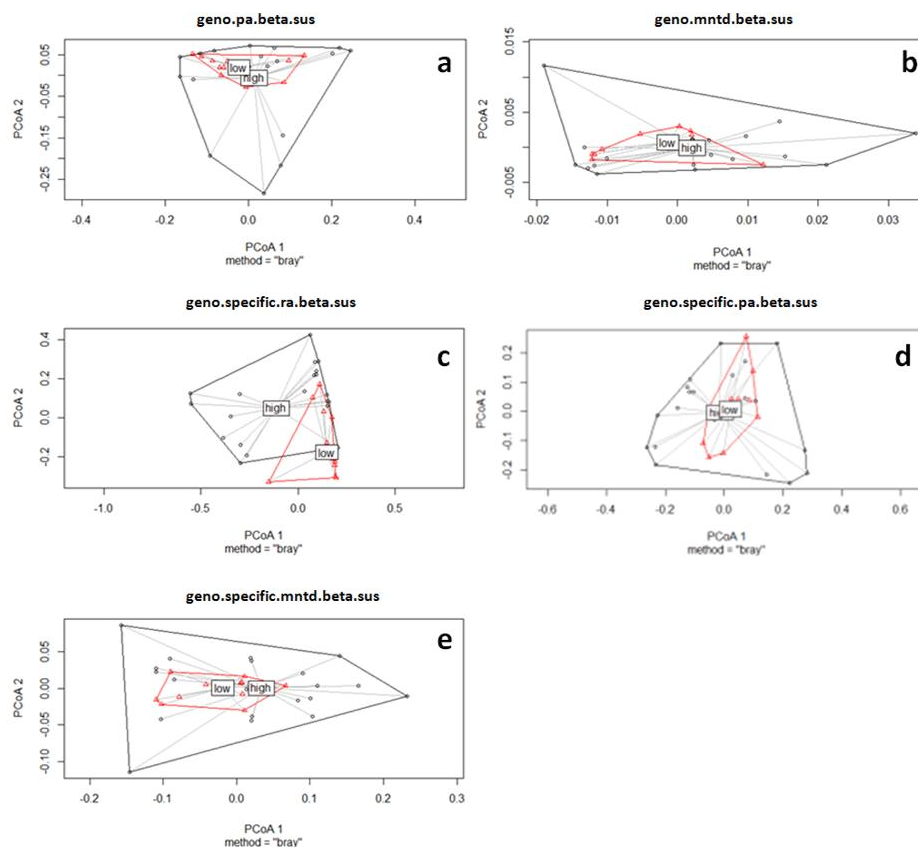
#### 4.2 Sampling consequences

However, differences in the bacterial communities occurring in the mucus of *Acropora cervicornis* may have resulted from drawbacks inherent to sampling methodology. Tips were sampled without regard to branch length or spatial orientation, and colonies were sampled from trees dispersed throughout the nursery for the genotype study. While depth differences were minimized to the extent possible, differences still existed. Bacterial communities within the SML of branching corals may be heterogeneous due to the coral's manifold structural configuration and the consequent differences in nutrient concentrations, hydrological regimes, irradiance exposures, and sedimentation levels imposing selective forces on microbial species (Kemp *et al.* 2015, Daniels *et al.* 2011). Indeed, differences in the relative abundance and presence/absence of water-coincident OTUs was partially explained by colony, suggesting small scale variations existed in the water regime between colonies. Furthermore, coral tissue and/or skeleton may have been inadvertently aspirated to varying extents along with the mucus. This may have been most important in the colony experiment since the surface was irritated with the blunt end of a syringe and not the less abrasive sterile glove. Consequently, downstream analysis was likely differentially impacted considering that the severity of contamination undoubtedly varied between samples. Coral tissues harbor PCR inhibitors that can restrict PCR adroitness and complicate comparisons by diminishing sample size. Furthermore, the PCR template has a proclivity towards DNA from cells with morphologies more

conductive to lysis during homogenization, as can be the case for coral cells. As such, PCR may then fail to adequately amplify certain bacteria as the primers nonspecifically bind to the mitochondria and chloroplasts within these cells. Additionally, extraction with the BiOstic kit (vs. the PowerLyzer Powersoil DNA extraction kit) for some genotypes may have led to higher, more diverse yields due to an additional lysis step (Weber *et al.* 2017).

#### 4.3 Anna Karenina principle

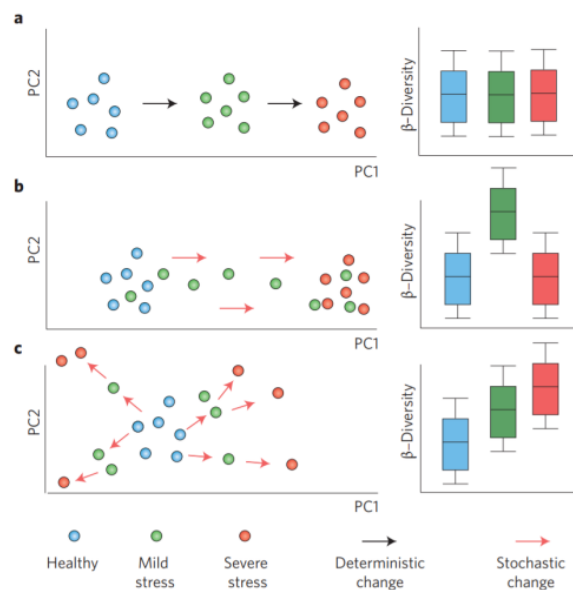
Mucus from different coral genotypes and disease-susceptibilities had significantly different mucosal microbiomes. Some caution must be taken when interpreting these results, as an assumption of PERMANOVA is equal variances and not all tests satisfied this requirement. One surprising finding was the significantly higher dispersion in genotypes vulnerable to disease, and this trend was detected in all betadisper statistics except for Bray-Curtis relative abundance [with water OTUs



**Figure 2.** Anna Karenina Principle as detected in high susceptibility genotypes for: a. PA Bray-Curtis for all genotype OTUs b. weighted UniFrac for all genotype OTUs c. RA Bray-Curtis for genotype-specific OTUs d. PA Bray-Curtis for genotype-specific OTUs and e. weighted UniFrac for genotype-specific OTUs.

included]. In general, the centroidal location of both high and low susceptibility groups coincided, but the variance for highly susceptible genotypes increased beyond that of genotypes with low susceptibility (Figure 2, Figure 3c).

These results are consistent with the Anna Karenina principle (AKP) (Zaneveld *et al.* 2017). It theorizes that the microbiomes of organisms no longer able to control their microbial communities [i.e. diseased] are unpredictably more diverse than the microbiomes of their healthy counterparts. The synergistic interactions of healthy hosts with their microbial associates limit those permitted in the microbiome, but deterioration of this regulatory ability can theoretically imperil host health by compromising immune systems (both innate and adaptive) and the advantageous effects of beneficial constituents. Under intensifying stress, microbiomes may transition into an alternate state by one of the following three scenarios: centroidal locations shifts but dispersion remains similar, centroidal location is displaced with dispersion increasing only when the host is intermediately stressed, or centroidal location is sustained but dispersion increases according to the severity of stress (Figure 3).



**Figure 3.** Schematic depicting possible microbiome transitions in organisms under increasing levels of stress A. Increasing stress displaces centroid location but dispersion remains unchanged. B. centroid location shifts, with dispersion increasing only under mild stress C. centroid location is similar to healthy individuals and dispersion increases along with increasing stress. (Image courtesy of Zaneveld, McMinds, and Vega Thurber (2017).

In the context of this experiment, genotypes of high disease susceptibility follow the latter scenario. The AKP may be more widespread than is currently documented,

but is either attributed to a byproduct of statistical analysis or is overlooked entirely. Indeed, the AKP was detected in the study conducted by Casey *et al.* (2015). The centroid location of weighted UniFrac distances obtained from corals suffering high mortality not only shifted from those of control and healthier corals as depicted in PCoA plots, but dispersion increased as well (Zaneveld, McMinds, and Vega Thurber 2017).

#### 4.4 *Thermally-derived constitutive mucus changes*

WBD is intimately linked with thermal anomalies arising from climate change, and epidemics manifest around 1-2 months post bleaching (Randall and van Woesik 2015). Therefore, disease resistance may be due in part to gene expression repertoires under thermal stress that allow the coral to sustain their symbiotic zooxanthellae. Vollmer and Kline (2008) documented full WBD resistance in three and high resistance in two of the 49 *A. cervicornis* genotypes, which was later correlated with the down-regulation of 70 kDa heat shock proteins. This in turn may signify thermal tolerance since changes in HSP activity were not as pronounced in heat-tolerant vs. heat-sensitive *A. hyacinthus* colonies (Librio and Vollmer 2016). Furthermore, coral genotypes can also influence symbiont behavior by differential gene expression, and those capable of regulating a suite of genes when stressed may confer a plasticity advantage to their symbionts by perpetuating sustainable conditions. Identical *Symbiodinium* strains harbored in six different *A. palmata* genotypes varied in their photochemical efficiency when colonies were cold-shocked. This was due to changing host gene expression, the magnitude of expression, and static expression [irrespective of treatment] that differed between genotypes (Parkinson *et al.* 2015). However, the microbiome determinants for *Seriatopora hystrix* suggested that coral genotype was not a contributing factor (Pantos *et al.* 2015).

Bleaching may initiate disease by altering the sugar constituents of the SML (Randall and van Woesik 2015, Lee *et al.* 2016), thereby giving pathogens a competitive advantage at the expense of commensals (Lee *et al.* 2016). Since zooxanthellae contribute 20-45% of net photosynthetic compounds as dissolved organic carbon or mucus to the polyp, zooxanthellae loss is likely deleterious to mucus vitality (Brown and Bythell 2005; Polson 2007). In the mucus of thermally stressed *A. muricata*, the relative saccharide proportions significantly changed at

higher temperatures; the temperature at which significance was detected varied by sugar (29°C and/or 31°C), with 31°C the temperature at which bleaching was identified with pulse amplitude modulated (PAM) fluorometry. These constitutive sugar changes were responsible for virtually half (46%) of the mucosal microbiome variability seen in treated individuals. *Gammaproteobacteria* comprised a substantial proportion of the microbiota at 26°C, but at 31°C they were displaced by *Alphaproteobacteria* and *Verrucomicrobiae*. Also, cyanobacteria increased (Lee *et al.* 2016). A rise in observed *Vibrio* spp. may have occurred despite the decreased levels of several sugars since *Vibrio* could exploit 75% of all sugars studied (Lee *et al.* 2016). Alternatively, that rise could have been linked to the denaturing of antibiotics produced by commensals adversely affected by heat or by the ability of pathogens to sequester beneficial species into sparse aggregates throughout the mucus. By segregating commensals, the positive impacts of these bacteria may be limited (Mao-Jones *et al.* 2010). Microbial composition changes may further modulate bacteria within the SML as one species' metabolite may be the metabolic substrate of another's (Ritchie and Smith 2004). Saccharides may also precipitate coral death if they are enriched beyond a threshold concentration. When lactose was enriched from 5 to 25 mg/L, *Montastraea annularis* suffered significant mortality. Intriguingly, moribund fragments displayed signs characteristic of disease, one of which included banding (Kuntz *et al.* 2005).

Nevertheless, Hadaidi *et al.* (2007) found no significant differences between the mucosal microbiome of healthy and visually-discerned bleached *Porites lobata*. Although the authors concede that bleaching likely changed the SML sugar agglomeration, they contend that the microbial similarities indicated mucus composition essentially remained constant. However, thermally-stressed corals in this study may have been inadvertently catalogued as healthy (Hadaidi *et al.* 2007) since over 50% of zooxanthellae must be lost before chromatic changes become apparent to the human eye (Miller *et al.* 2014).

#### 4.5 Seasonal sediment effects

Time of sampling did not affect the sediment microbiome for the nearshore to offshore transect sites, and this may have been due to the close temporal proximity in which sediments were collected (approximately a month and a half). When sediments

were collected in different seasons in the Red Sea, winter samples were more unique than those collected in the fall and summer, and these differences were attributed to nutrient influxes emanating from wind-forced upwelling waters (Schottner *et al.* 2011).

#### 4.6 Biotic and abiotic sediment microbiome influences

Location was significant for all statistics involved. If bacteria are universally-distributed, spatial heterogeneity in their communities may be due to abiotic and biotic factors distinctive to each location selecting for which species become residents (Green and Bohannan 2006). Tavernier Creek delineates Key Largo and Plantation Key, with a benthos of *Thalassia* sp. rooted in a substrate composed predominately of calcareous *Halimeda* sand particles. The benthos then progresses into a rocky ledge whose sandy shoreline boundary is inhabited by *Thalassia testudinum* patches adjacent to exposed Pleistocene limestone. Abutting this bedrock is Hawks Channel, whose bottom consists of a micritic seagrass bed (mostly *Thalassia testudinum*) of *Halimeda* and mollusc particles. The seagrass bed advances beyond Hawks Channel, but the micritic substrate is replaced by the carbonate sands of *Halimeda trident* and *H. opuntia* remnants. Eventually the benthos transitions to patches of strictly carbonate sand, grains of which originate from the skeletons of molluscs, corals, byrozoans, and sea urchin spines. It is within these sandy patches that the Coral Restoration Foundation's Tavernier Nursery is located, just west of the coral rubble flanking the severely deteriorated Pickles Reef (Lidz *et al.* 2006). Alternatively, differences may be attributed to constrained dispersion (Green and Bohannan 2006).

A sediment transect in Australia revealed that sediment microbial communities elicited different assemblages on both large (20 m) and small (10 cm) spatial scales. Since grain size and levels of primary production were similar along the transect, differences in community composition amongst Australian sites may have been due to the unique topography inherent to each site. By affecting water dynamics, these topographical profiles determine in part the intensity of hydrological forces acting upon the substrate. Water currents determine the stability of the substrate, and wave-induced sediment upwelling exposes grains to oxygen and detritus while simultaneously flushing away nutrients. Theoretically, laminar flow over nearshore sediments should provide a more stable environment than the wave-swept reef crest.

Accordingly, the reef crest may be less diverse due to high disturbances selecting for fewer species that are well-adapted to fluctuating conditions. Indeed, overall richness generally increased from the reef crest to the shoreline in Australia (Hewson and Fuhrman 2006). In this study, no trend in increasing richness was observed from Pickles Reef rubble patch to Tavernier Creek.

Sediment microbiomes may also be influenced by the size of its particle constituents (Schottner *et al.* 2011). Carbonate grains are common to each of these sites (Lidz *et al.* 2006), and spatial differences in microbiomes suggest particle size may have differed between each of the sites. Carbonate sands are comprised of coarsely grained (500–1000  $\mu\text{m}$ ) calciferous skeletons with a porous topography. This complex topography equates to a higher specific surface area and greater permeability than sands composed of smooth, terrestrially-derived silicate particles with a size of 250–500  $\mu\text{m}$ . The spatially intricate microcosm affords greater protection, substrate access, and biofilm production. Studies have produced conflicting results regarding whether or not bacterial numbers are equivalent between differing sand size fractions. Carbonate sands conceivably have a greater number of bacteria due to a larger specific surface area, but the greater numbers afforded by this trait may be offset by its large volume. In the Red Sea, sand particle size significantly structured the microbial reef sediment community, as did season and depth. However, the influential magnitude (and differential dominance) of seasonality and depth were dictated by grain size (Schottner *et al.* 2011).

#### 4.7 *Effects of sedimentation on coral*

Sand-size particles are generally characteristic to offshore regions, whereas silt-sized particles are characteristic to inshore regions. Consequently, sedimentation stress may be a function of sediments' shore proximity (Weber *et al.* 2006). At 22 reefs situated around 11 Caribbean Islands, the proportion of terrigenous sediment was inversely correlated with coral cover, and which species of coral was present significantly depended on whether sites had a low (0–14%) or high (29–95%) abundance of terrestrial sediment. Yet of the fifteen coral species contributing to almost all of the observed differences between these sites, only one was significantly affected when the percentage of terrestrial sediment was considered. However, the amount of fine-grained sediment may be a poor indicator of the magnitude of



terrigenous input since no significant proportional correlation was found between the two in samples collected from the Caribbean Islands of Saint Lucia and Saba. Classification of the proportion of fine-grain sediment (low: 0-0.8% vs. high: 2-18%) didn't significantly affect which coral species were present, although this may be due to the fact that the amount failed to reach a damage-provoking threshold (Begin, Wurzbacher and Cote 2013).

Particle size also determines the severity of sedimentation damage to corals due to the corresponding extent of adsorbed nutrients. Silts adsorb more microorganisms and particulates than sand particles due to their larger surface area to volume ratio, and are thus also more adhesive. This adhesive propensity, along with a thicker settling layer, may explain why *Montipora peltiformis* fragments were more effective at eradicating nutrient-poor, sandy sediments. Furthermore, silt negatively impacted photosynthetic yield of this coral species, with maximum stress precipitating in less than two days; fine and medium - sized sands had no demonstrable effect on yield. However, although sands did not photophysiologicaly stress treated corals, bleaching was later apparent and none of the treated corals returned to baseline conditions (Weber *et al.* 2006).

Coral mortality from sedimentation may derive from increased bacterial growth arising from adsorbed organic matter, zooxanthellae expulsion [due to anoxia stemming from photosynthetic obstruction], and/or hydrogen sulfide resulting from anoxic sulfate reduction. Yet Weber *et al.* (2012) discovered that the cause of mortality in *M. peltiformis* was anoxia and increased acidity stemming from microbial respiration of organic-enriched (0.3% and 0.6%) sediments. H<sub>2</sub>S concentrations only became toxic upon respiration of necrotic tissue and mucus, and although H<sub>2</sub>S accelerated the mortality rate, death precipitated in its absence. Photosynthetic yield, necrosis, and H<sub>2</sub>S were significantly affected by both exposure time and the concentration of organic carbon within the sediments, whereas oxygen and pH were solely dependent on the organic carbon concentration. If corals ferment in anoxic conditions, death may eventually result through increased cell acidification. Alternatively, cells may lose control of their pH if fermentation-acquired energy doesn't offset the energy required to sustain cellular pH. Sufficient carbon enrichment may also favor the growth of certain bacterial groups to the detriment of others. Cloning of the 16S rRNA gene revealed that sediments enriched with 0.6% organic

carbon supported the least diverse microbial community after a 3 hour exposure, with Gammaproteobacteria accounting for 90% of the approximately 120 clones sequenced. The authors of this study argue that it is not infectious agents that cause destruction, but bacteria specifically within the sediments: anoxia in combination with decreased pH generated damage in the absence of nutrient enrichment and sediment exposure, microbial activity increased in the sediments but not at the sediment-tissue interface, and DGGE bands of coral-exposed sediments coincided with those of the control sediments (Weber *et al.* 2012).

## CHAPTER 5: CONCLUSIONS

Microbiome differences between colonies may be due to micro-scale environmental discontinuities resulting from its complex branching structure. Alternatively, varying amounts of aspirated tissue between samples may have interfered with PCR integrity and thus produced false differences. Differences were also detected between genotypes of different disease vulnerabilities, suggesting that genotypes with low disease susceptibility employ different microbiome regulation mechanisms than those exhibiting high disease susceptibility. Most notably, genotypes with high susceptibilities conformed with the Anna Karenina Principle. While their centroidal location was similar to genotypes resistant to disease, the higher dispersion they exhibited suggests a possible loss of regulatory control over microbiome constituents. This loss may be the consequence of differential gene regulation under periods of heightened thermal stress that then affect zooxanthellae health. Loss of zooxanthellae integrity and/or numbers may change the sugar composition within the mucus, culminating in a detrimental transformation of the mucus microbiome. However, genotypic differences could also be attributed to the use of different DNA extraction kits. For sediments, location was the only driving factor regulating microbiome composition. Although not quantified for this study, these spatial differences may have been due to differences in grain size between sites. Time of sampling was not a factor, and this may have been a consequence of the relatively short interval between sampling timepoints. Particle size, in conjunction with the amount of adsorbed nutrients, may determine the severity of damage to sediment-laden corals.

Thus, this study suggests coral mucus is highly variable (especially with genotypes vulnerable to disease) and is influenced by both regulatory control by the coral host and ambient environmental conditions.

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